and more or less electron dense, while others exhibited various stages from a homogeneous appearance to the striking wheel-like arrangement (Fig. 3). Quite frequently type I cells were so laden with inclusions that their cytoplasm bulged out locally or their nuclei were indented. An increased number of free cells in the alveolar space was also observed. These cells were often arranged in groups of three or more and their cytoplasm revealed numerous inclusion bodies of various electron densities, closely resembling the homogeneous inclusions in type I cells. Up to 2 days after the injection there was no apparent change in the number and appearance of the cytoplasmic bodies in type I cells. At 3 days and thereafter, very few of these bodies were seen and the cytoplasm of type I cells appeared indistinguishable from that of control animals.

These observations strongly suggest that the type I alveolar lining cells participate in the clearing mechanism of the lung tissue. Such function, thus far, has never been attributed to these cells but rather to a variety of other cells in the lung tissue, including the type II cells. Although much additional study is necessary to elucidate the nature of these cytoplasmic bodies, as well as the molecular events and control mechanisms responsible for the activity exhibited in the type I pneumocytes, the observations reported here may well initiate a revision of our present concept of the still poorly understood clearing mechanism in lung tissue.

EDITH J. HAPKE
HARLAN J. PEDERSON
Research Services, Wood Veterans Administration Center, and *Department of Medicine, Marquette School of Medicine, Milwaukee, Wisconsin*

References and Notes

1. F. N. Low, *Anat. Rec.* **117**, 241 (1953).
2. Albumotope-LS, aggregated radio-iodinated (I^{131}) (albumin) (human); Squibb Division, Olin Mathieson Chemical Corp.
3. H. N. Wagner, Jr., D. C. Sabiston, Jr., M. Iio, J. G. McAfee, J. K. Meyer, J. K. Langan, *J. Amer. Med. Ass.* **187**, 601 (1964).

21 May 1968

Pesticide Transformations: Production of Chloroazobenzenes from Chloroanilines

Abstract. *Aniline and 11 different chloroanilines were added to soil. No azo compound was formed from aniline, but all monochloro- and some dichloroanilines were transformed to their corresponding dichloro- and tetrachloroazobenzenes. Other dichloroanilines and the trichloroanilines were stable in soil. Peroxidase catalyzed the formation of azo compounds by some chloroanilines. Correspondence in the range of substrates used and products formed in the two systems suggests a peroxidatic mechanism for the synthesis of azo compounds from anilines in soil.*

It was recently demonstrated that an aniline intermediate in the decomposition of a pesticide was condensed to form an azo compound; specifically the herbicide propanil (3′,4′-dichloropropionanilide) was degraded microbiologically in soil to 3,4-dichloroaniline, two molecules of which were oxidatively linked as 3,3′,4,4′-tetrachloroazobenzene (*1*). Since anilines are known to be produced during decomposition of various other substances, including phenylcarbamate, phenylurea, and acylanilide herbicides (*2*), formation of azo compounds may be a common rather than a rare phenomenon, and studies of the requirements and mechanism for aniline-condensation reactions appeared warranted. We now describe the influence of molecular configuration on the ability of anilines to be transformed to azo compounds in soil, and present evidence that the reaction is catalyzed by the enzyme peroxidase. The work relates directly to questions of environmental pollution, and is of concern to public health and welfare since some azo compounds are carcinogenic (*3*).

Formation of azo compounds in soil was investigated by mixing 50 mg of each of several different anilines separately with 50 g (dry weight) of Nixon sandy loam (*p*H 5.5). The soil samples were moistened to 60 percent of capacity, incubated in covered beakers at 27°C for 14 days, and then extracted with acetone. The extracts were concentrated and subjected to gas-chromatographic analysis with a flame ionization detector; the stainless steel column was 1.8 m long by 3 mm in outside diameter and packed with 5 percent UC-W98 on Chromosorb W. A portion of each extract was evaporated to dryness, and the residue was dissolved in methanol and examined spectrophotometrically with a Beckman-DB instrument.

Peroxidatic formation of azo compounds was measured in a system containing 50 mg of an aniline substrate in 50 ml of 0.2*M* acetate buffer at *p*H 5, 0.1 ml of a 30-percent solution of H_2O_2, and 13.5 units of peroxidase (Sigma Chemical Co.; horseradish peroxidase type II, 135 purpurogallin units per milligram). Additional increments of H_2O_2 and peroxidase were supplied at each of three consecutive 30-minute intervals, and the reaction mixture was stirred magnetically for a total of 2 hours. The system was then extracted with three 20-ml quantities of hexane, which were combined, concentrated, and analyzed for azo compounds by the gas-chromatographic procedure used for examination of the soil extracts.

All commercial chemicals were examined for homogeneity by gas chromatography. Chlorinated anilines were purified by repeated recrystallization from ligroin. Chlorinated azobenzenes were synthesized by oxidation of appropriate chloroanilines or by reduction of their corresponding nitrobenzenes (*4*).

Our concern was with the detection and identification of azo compounds as a means of understanding the requirements for and mechanism of their formation in soil. All analyses were qualitative, with no attempt to measure either the rate or extent of a transformation. Products other than azo compounds were not isolated or identified. No azo compounds were detected in soil that was untreated or in soil that was sterilized and treated with filter-sterilized aniline solutions (*1*). Moreover, there was no transformation of anilines to azo compounds in buffered H_2O_2 solutions unless the reaction was catalyzed by peroxidase.

The 12 anilines employed by us are illustrated in Fig. 1 along with the azo compounds that were formed from the anilines in soil and by peroxidase. In every instance the identification of an azo compound was based on two independent characteristics: its retention time and absorption spectrum.

Aniline itself was transformed in soil and by peroxidase, but no azo compound was among the products. The expected dichloroazobenzene was produced enzymically and in soil from each of the three monochloroanilines.

Some dichloroanilines (2,3-; 2,4-; 3,5-substitution) were oxidized to azo compounds in soil only, one (3,4-substitution) was transformed to tetrachloroazobenzene in both soil and enzyme solution, and two dichloroanilines (2,5-; 2,6-substitution) resisted change completely. Since the trichloroanilines were also recalcitrant, one may conclude that chlorination of the 2,5- or 2,6- positions stabilized the molecule and protected aniline from biochemical transformation. Alternatively, absence of substituents at the 2,5- or 2,6- positions was required for the transformation of chloroanilines to azo compounds.

Of particular note was the fact that 2,3-dichloroaniline was converted to 3,3′-dichloroazobenzene instead of to the expected tetrachloroazobenzene. Attempts at laboratory synthesis of 2,2′,3,3′-tetrachloroazobenzene provided chemical evidence that under certain conditions a chlorine substituent, adjacent to both a nitrogen and an additional chlorine substituent, is labile and eliminated during azo condensation.

The reported (*5*) catalysis by peroxidase, of the synthesis of 4,4′-dichloroazobenzene from monochloroaniline, suggested that a peroxidatic mechanism may be responsible for production of azo compounds in soil. Soil extracts catalyze peroxidation of pyrogallol (*6*). Nixon sandy loam had peroxidase activity, and the similarity in specificity for aniline substrates, exhibited by the soil and by peroxidase, was impressive (Fig. 1). The four compounds that were stable in soil were also resistant to peroxidatic change, and seven of the eight remaining substances were transformed in both soil and the perixodase systems. The single exception to complete correspondence in reactivity was 3,5-dichloroaniline which was refractory to peroxidase but oxidized to an azo compound in soil.

The utilization of different anilines as substrates by soil and peroxidase was more highly correlated than was the ability of these systems to produce azo compounds. Four chloroanilines gave rise to azobenzenes that were identical in both soil and enzyme solution. Three more anilines were oxidized to azobenzenes in soil, but no azo compounds were detected among the products of their peroxidation (Fig. 1). Since these differences may be reduced or eliminated completely by the definition and use of conditions that are optimal for the reaction, investigations based on a peroxidatic mechanism for the production of azo compounds in soil now seem justified.

One may use the results summarized in Fig. 1 as a basis for prediction that pesticides partly composed of a chloroaniline unsubstituted in both ortho (2,6-) positions, or in adjacent ortho and meta (2,3- or 5,6-) positions, are likely to give rise to azo compounds during decomposition in soil. Experiments with Dicryl, Karsil, and propanil provided limited support for this proposal; each has a 3,4-dichloroaniline moiety, a substantial portion of which was liberated and condensed to 3,3′,4,4′-tetrachloroazobenzene in soil (*7*). Since chloroanilines are important parts of many different pesticides, one may anticipate that their fate will be further tested in this and other laboratories; our prediction may be further supported, and some exceptions may be identified and described. Ultimately a principle should be established that relates chemical configuration to formation of azo compounds and serves as a guide for the synthesis of useful pesticides that undergo biochemical degradation without producing undesirable residues in soil.

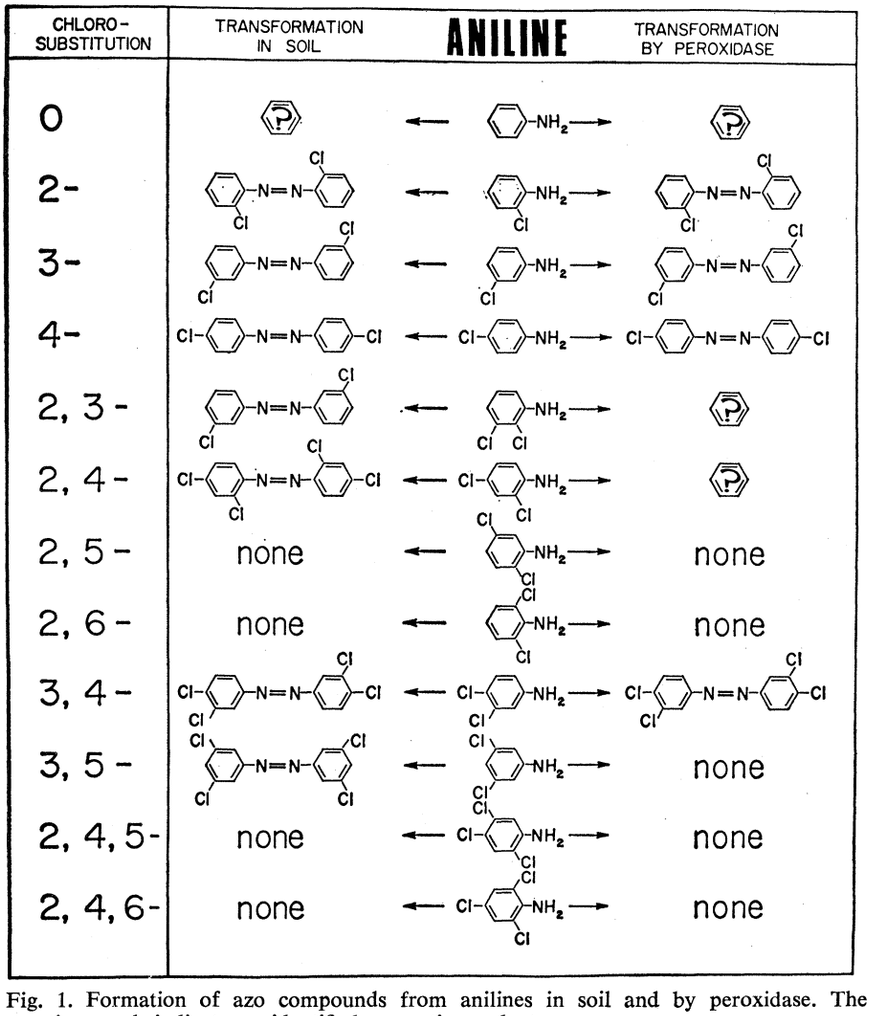

Fig. 1. Formation of azo compounds from anilines in soil and by peroxidase. The question mark indicates unidentified aromatic products.

RICHARD BARTHA, H. A. B. LINKE
DAVID PRAMER
Department of Biochemistry and Microbiology, Bureau of Conservation and Environmental Science, Rutgers University, New Brunswick, New Jersey

References and Notes

1. R. Bartha and D. Pramer, *Science* **156**, 1617 (1967).
2. H. Geissbühler, C. Hasselbach, H. Aebi, L. Ebner, *Weed Res.* **3**, 277 (1963); R. L. Dalton, A. W. Evans, R. C. Rhodes, *Weeds* **14**, 31 (1966); D. D. Kaufman and P. C. Kearney, *Appl. Microbiol.* **13**, 443 (1965); R. Bartha, R. P. Lanzilotta, D. Pramer, *Appl. Microbiol.* **15**, 67 (1967).
3. J. H. Weisburger and E. K. Weisburger, *Chem. Eng. News* **44**(6), 124 (1966).
4. The preparation, purification, and chemical properties of azo compounds will be described in detail elsewhere.
5. D. G. H. Daniels and B. C. Saunders, *J. Chem. Soc. London* **1953**, 833 (1953).
6. A. S. Galstyan, *Dokl. Akad. Nauk Arm. SSR* **26**, 285 (1958).
7. R. Bartha, *J. Agr. Food Chem.*, in press.

8. Aided by PHS research grant ES-16. We thank the Upjohn Co. for donating 2,4,6-trichloroaniline. A paper of the Journal Series, New Jersey Agricultural Experiment Station.

31 May 1968